Choosing the best wavelength for detection of peptide maps

Biopharmaceutical

Peptide mapping is one of the most important and widely used techniques in protein characterization. It is used for quality control of recombinant protein batches (fingerprint analysis) and to get structural information of new isolated proteins of known or unknown function. Proteins are cleaved chemically or enzymatically with proteases of high or less high cleavage specificity, resulting in peptide maps which are characteristic for each individual protein.

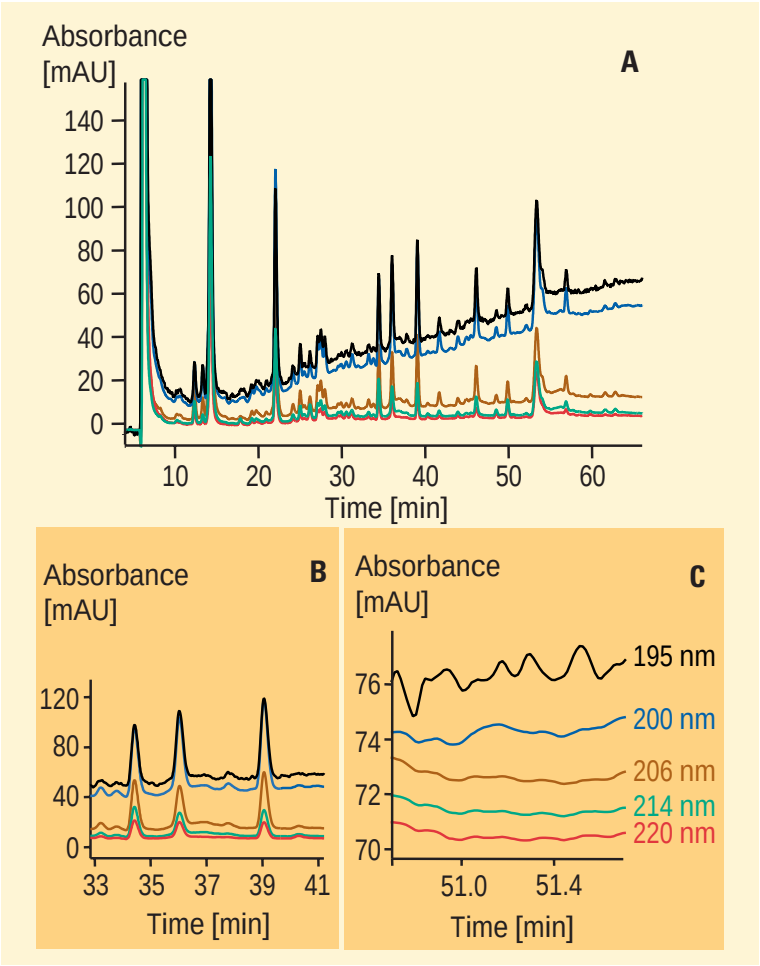


Figure 1
Separation of a 2-pmol tryptic peptide map on a 300-µm id C-18 capillary column. A=overlay of the recorded traces at different wavelengths

Conditions

Column
0.3 x 250 mm Vydac C-18 (LC Packings, The Netherlands)
Mobile phase
A = 0.025 % TFA
B = acetonitrile, 0.020 % TFA
Flow rate
0.1 ml/min with flow split to 4 µl/min
Flow splitter
VAR-100 (LC Packings, The Netherlands)
Gradient
0–5 min 2 % B
5–70 min, 2–45 % B
UV detector
diode array detector
195 nm, 200 nm, 206 nm, 214 nm, 220 nm, each with 10-nm bandwidth
spectra 200–320 nm
500 nl flow cell kit
Temperature
ambient

Peptide maps are typically separated on reversed-phase columns, mostly on C-18 columns using either standard HPLC or capillary HPLC for higher sensitivity. After application to the colum, peptides stick to the C-18 material and are then gradually eluted by running a gradient from an aqueous phase to an organic phase. Standard HPLC solvents are water as solvent A and acetonitrile (sometimes methanol and isopropanol) as organic solvent B. TFA (trifluoroacetic acid) is added to the HPLC solvents as organic modifier. Routinely, 0.1 % or 0.05 % TFA are added. Depending on quality and age of TFA, rising baselines and increased pump pulsations are well-known negative phenomena which make the interpretation of weak peptide maps often difficult.

To detect peptide maps wavelengths at 220 nm 214 nm are mainly used. However, these wavelengths are not the most sensitive ones, because the absorption maximum of all amino acids is below 210 nm.

Here, we compare the detection sensitivity of peptide maps at 220, 214, 206, 200 and 195 nm. We also evaluate the baseline and the baseline noise using 0.025 % TFA in solvent A and 0.02 % TFA in solvent B, a TFA concentration which is a sufficient organic modifier amount. Additonally, this reduced TFA amount influences the sensitivity of on-line LC-MS analysis positively.

Figure 1 part A show the separation of a 2-pmol tryptic peptide map on a C-18, 300-mm id capillary column. Flat baselines are obtained using 220 nm and 214 nm detection. Detection at 206 nm also results in an almost completely flat baseline. Detection at 200 and 195 nm, however, show significantly rising baselines.

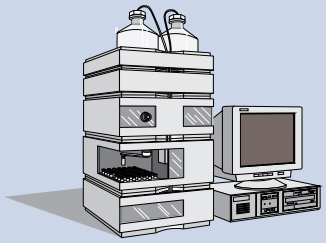
Figure 1 part B show sensitivity. Detection at 214 nm is about 30 % more sensitive than at 220 nm whereas at 206 nm the gain in sensitivity is more than 100 % compared to 220 nm. Slightly increased signal heights are further obtained at 200 nm and at 195 nm. However, at these wavelengths, especially at 195 nm, the baseline noise is significantly higher than at 206, 214 and 220 nm (showing comparable baseline noise) so that overall no sensitivity increase at 195 nm and 200 nm is achievable. This is shown in figure 1, part C.

Hence, the best wavelength for the detection of peptide maps is 206 nm, which provides a flat baseline and maximal sensitivity.

Equipment

Agilent 1100 Series

- Binary pump (includes vacuum degasser)
- Autosampler
- Diode array detector capillary flow cell 10-mm path length, 500 nl cell volume
- Agilent ChemStation + 3D software



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